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Effect of Essential Oils and Isolated Compounds from *Pimpinella* Species on NF-κB: A Target for Antiinflammatory Therapy

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***Pimpinella* essential oils and isolated compounds were screened for their inhibitory activity against NF-κB mediated transcription in SW1353 cells. Twelve oils were effective in inhibiting NF-κB mediated transcription. Especially the roots of *P. corymbosa*, *P. tragium* and *P. rhodanta* showed potent activities with IC_{50} values of 2, 3 and 6 μg/mL, respectively. Five pure compounds, 7 (4-(2-propenyl)phenylangelate), 12 (4-(3-methyloxiranyl)phenyltiglate), 17 (4-methoxy-2-(3-methyloxiranyl)phenyl isobutyrate), 18 (4-methoxy-2-(3-methyloxiranyl)phenylangelate) and 21 (epoxy pseudoisoeugenol-2-methylbutyrate) inhibited NF-κB mediated transcription with IC_{50} values of 5.5, 1.2, 0.01, 3.6 and 11 μg/mL, respectively. None of the compounds were cytotoxic to mammalian cells. These findings add significant information to the pharmacological activity of *Pimpinella* species and their beneficial effects and use in disease prevention especially those related to inflammation. Copyright © 2007 John Wiley & Sons, Ltd.**

Keywords: Pimpinella species; phenylpropanoid; essential oil; NF-κB mediated transcription.

INTRODUCTION

The family Umbelliferae (Apiaceae) is a large family containing 300–455 genera and over 3000 species. Several Umbelliferae genera are used intensively in industry because of their properties as aromatic and medicinal plants (Baser, 2002). Especially anise (*Pimpinella anisum* L., Umbelliferae) has been valued highly since ancient times and it is widely cultivated in Europe, Balkans, North Africa, Asia and South America as an aromatic spice crop. The aniseed is of economical importance as a flavoring agent in food and perfumery industries (Baser, 1997, 2002; Tabanca *et al.*, 2003). The aniseed also has been credited with a long list of traditional medicinal uses: carminative, antiseptic, antispasmodic, expectorant, diuretic, diaphoretic, stimulant and stomachic (Tabanca *et al.*, 2003, 2004, 2005a, 2005b). In the Himalayas, the ethanol extract of *P. diversifolia* DC. seeds, has been reported to be a strong fungitoxic and the extract of the whole plant has been found to possess spermicidal activity in rat semen (Bottini *et al.*, 1986). The hot aqueous extract of the root of *P. tirupatiensis* Balakr. & Subram. is known as an aphrodisiac and used for peptic ulcers in India (Nagaraju and Rao, 1990). In Turkey, *P. anisetum*, an endemic species, has been used for smoking to promote expectoration and many *Pimpinella* species have been used as animal feed to increase milk secretion (Tabanca *et al.*, 2003). Recently, the estrogenic activity of isolated compounds and essential oils of different *Pimpinella* species were reported. Of the pure compounds, only (*E*)-anethole showed estrogenic activity with an EC_{50} of 625 μg/mL. Some essential oils were found to be estrogenic despite the absence or trace amounts of anethole. The study indicated that components other than anethole could also contribute towards the estrogenic activity (Tabanca *et al.*, 2004).

Both the extracts and essential oils of *Pimpinella* are known to have a high content of pseudoisoeugenol-type phenylpropanoids which is unique to the genus (Kubeczka, 1997). Our earlier investigations performed on *Pimpinella* species resulted in the isolation of four new and 18 known compounds (Tabanca *et al.*, 2003, 2004, 2005a). The antimicrobial, antifungal and antimalarial activities of some of these compounds were reported (Tabanca *et al.*, 2005a).

Epidemiological studies have demonstrated that nonsteroidal antiinflammatory drugs (NSAIDs), which are also potent cyclooxygenase (COX) inhibitors, mediate cancer preventive and tumor regressive effects in the human colon. Recent studies have also shown that inhibition of NF-κB activation results in the prevention of colon cancers (Rayyan *et al.*, 2002; Scaife *et al.*, 2002). The nuclear factor kappa B (NF-κB) is an inducible, ubiquitous transcriptional regulator. It acts as a central

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mediator of the human immune response and regulates the expression of a variety of genes involved in immune and inflammatory response, including the COX-2 gene (Wulczyn *et al.*, 1996; Baldwin, 1996). It has been reported to be involved, at least in part, in the antiinflammatory action of NSAIDS that inhibit the phosphorylation of NF-κB (Yin *et al.*, 1998). Specific blockade of NF-κB signaling may be beneficial to inflammatory diseases, and thus NF-κB seems to be an important target for antiinflammatory therapy.

As part of our continuing efforts to study medicinal plants, the antiinflammatory and cytotoxic activities of essential oils from different plant parts of various *Pimpinella* species and isolated compounds were investigated to explore the beneficial effects of this species.

MATERIAL AND METHODS

Plant materials. Plant materials were collected from different parts of Turkey and voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University in Eskisehir (ESSE) (Tabanca *et al.*, 2003, 2004, 2005a).

Isolated compounds. Compounds **1–21** [*trans*-anethole (**1**), methyleugenol (**2**), *trans*-isoosmorhizole (**3**), dictamnol (**4**), 4-(6-methyl-bicyclo[4.1.0]hept-2-en-7-yl)-butan-2-one (**5**), 4-(1-propenyl)phenylisobutyrate (**6**), 4-(2-propenyl)phenylangelate (**7**), 4-(1-propenyl)phenyl tiglate (**8**), 4-(1-propenyl)phenyl-2-methylbutyrate (**9**), alismol (**10**), 1-methyl-4-(5-methyl-1-methylene-hex-4-enyl)-7-oxa-bicyclo[4.1.0]heptane (**11**), 4-(3-methyloxiranyl)phenyltiglate (**12**), 4-(3-methyloxiranyl)phenyl-2-methylbutyrate (**13**), 4-methoxy-2-(1-propenyl)phenyltiglate (**14**), 4-methoxy-2-(1-propenyl)phenylangelate (**15**), pseudoisoeugenol-2-methylbutyrate (**16**), 4-methoxy-2-(3-methyloxiranyl)phenyl isobutyrate (**17**), 4-methoxy-2-(3-methyloxiranyl)phenylangelate (**18**), 4-methoxy-2-(3-methyloxiranyl)phenyltiglate (**19**), 12-hydroxy-β-caryophylleneacetate (**20**), epoxy pseudoisoeugenol-2-methylbutyrate (**21**)] were isolated from essential oils of *Pimpinella* species which were reported previously (Tabanca *et al.*, 2003, 2004, 2005a). All the compounds were identified based on their spectral data (1D- and 2D-NMR, GC/MS and HR-ESI-MS) (Tabanca *et al.*, 2003, 2004, 2005a).

Reporter gene assay for inhibition of NF-κB mediated transcription. Human chondrosarcoma cells (SW1353) were cultured in a 1:1 mixture of DMEM/F12, supplemented with 10% FBS and 100 U/mL penicillin G sodium and 100 μg/mL streptomycin. The nuclear factor-κB (NF-κB) reporter construct contained two copies of the element from the immunoglobulin K promoter (p BIIXLUC) and was a gift from Dr Riccardo Dalla-Favera. The Sp-1 reporter plasmid (pGL3-promoter) was obtained from Promega. The assay was performed as described previously (Ma *et al.*, 2006). Briefly, luciferase plasmid construct (25 μg) was added to the cell suspension (1.2×10^7 cells in 500 μL) and incubated for 5 min at room temperature. The cells were electroporated at 160 V and one 70 ms pulse in a BTX Electro Square Porator T 820 (BTX I, San Diego, CA). Transfected cells were added to the wells of a 96-well plate (1×10^5 cells/well) in 200 μL DMEM/F12 (supplemented with 10% FBS and antibiotics). After 24 h, the cells were exposed to different concentrations of test samples for 30 min and then induced with PMA (phorbol myristate acetate, 70 ng/mL) for 8 h for the activation of NF-κB. After removing the medium, the cells were lysed by adding 40 μL of a 1:1 mixture of lucLite reagent and PBS containing 1 mM calcium and magnesium (Packard Instrument Company, Meriden, CT). Light output was detected in a TopCount microplate reader in a single-photon counting mode (Packard).

Cytotoxicity. Cytotoxicity to Vero cells (monkey kidney fibroblast) and solid tumor cells (SK-MEL, SK-OV3, BT-549 and KB) was determined as described previously using the neutral red assay procedure (Tabanca *et al.*, 2003).

RESULTS AND DISCUSSION

Earlier investigations performed on the essential oils of *Pimpinella* species resulted in two new (**7**, **13**) and 14 known phenylpropanoids, two new (**5**, **11**) and four known sesquiterpenes (Fig. 1). The structures of the compounds were determined previously from 1D-, 2D-NMR and MS experiments (Tabanca *et al.*, 2003, 2004, 2005a). This paper describes antiinflammatory and cytotoxic activities of these compounds isolated from *Pimpinella* species.

The essential oils from different plant parts of various *Pimpinella* species and isolated compounds were tested for their inhibitory activity against NF-κB dependent transcription induced by PMA in SW1353 cells. Using NF-κB activation as a molecular target, the essential oils and pure compounds obtained from different *Pimpinella* species were screened for the first time. The results are shown in Fig. 2, Tables 1 and 2. Human chondrosarcoma cells were transiently transfected with NF-κB promoter plasmid. At 24 h after transfection, the cells were exposed to different concentrations of essential oils and compounds for 30 min and then incubated with PMA for an additional 8 h for induction of NF-κB mediated transcription which is measured in terms of luciferase expression. A luciferase construct with binding sites for Sp-1 was used as a control because this transcription factor is unresponsive to inflammatory mediators such as PMA. Hence, the measurement of Sp-1-mediated luciferase expression is useful for detecting agents that nonspecifically inhibit luciferase expression because of cytotoxicity, inhibition of luciferase enzyme activity or light output (Ma *et al.*, 2006). The main components of the essential oils were reported previously on the basis of their GC-MS analyses (Tabanca *et al.*, 2004). Inhibition of NF-κB transcription mediated by the aforementioned oils is shown in Table 1 as IC_{50} values. The highest activities amongst the oils were observed with the roots of *P. corymbosa* Boiss. (IC_{50} = 2 μg/mL), *P. peucedanifolia* Fisch. ex Ledeb. (IC_{50} = 3 μg/mL) and *P. rhodantha* Boiss. (IC_{50} = 6 μg/mL) followed by the roots of *P. tragium* Vill. ssp. *polyclada* (Boiss. et Heldr.) Tutin (IC_{50} = 11 μg/mL) and the aerial parts without fruits of *P. peregrina* L. (IC_{50} = 27.5 μg/mL). None of the oils inhibited Sp-1

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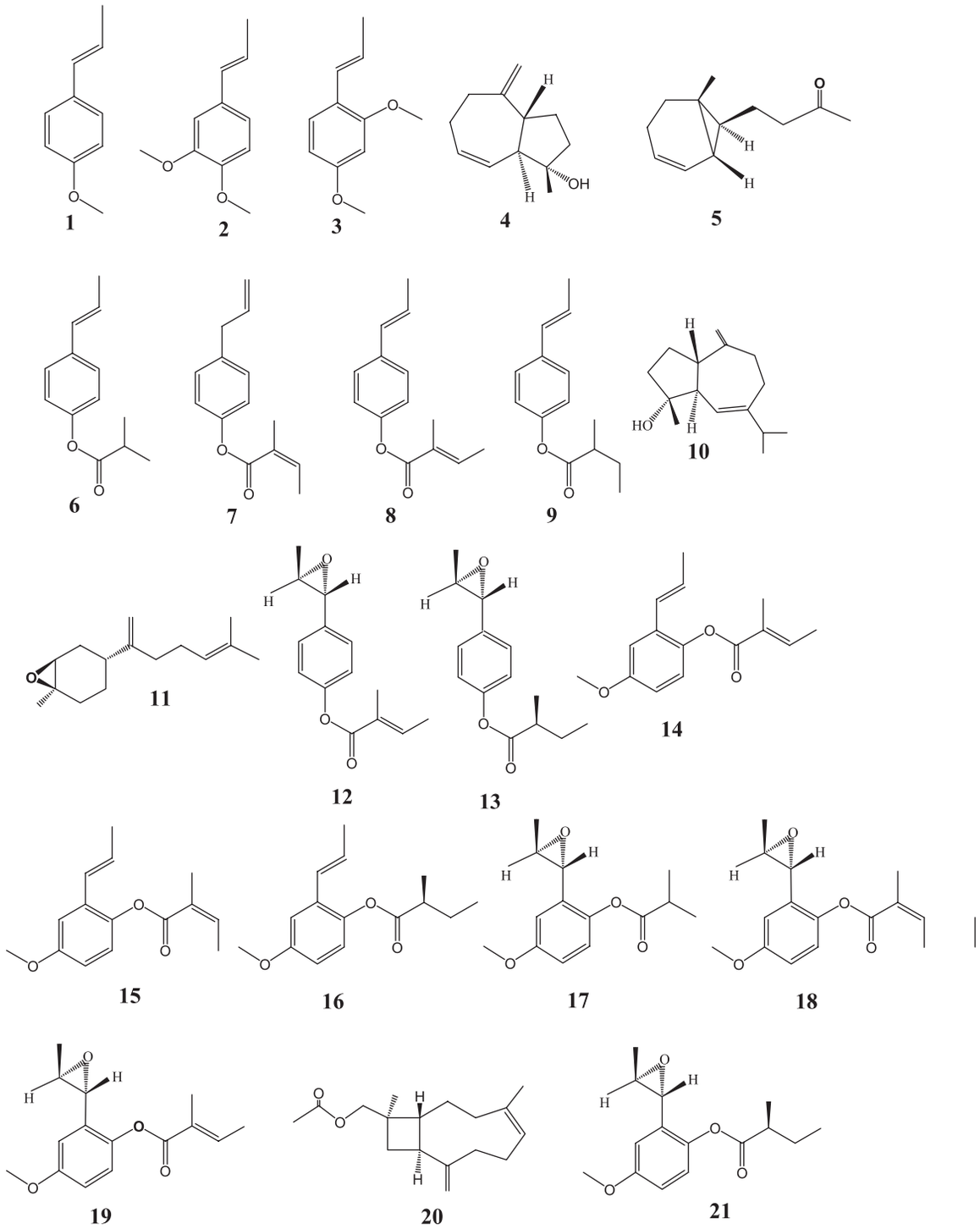

Figure 1. Structure of the compounds.

dependent luciferase expression indicating that their effect on NF-κB was specific.

Of 21 pure compounds, only **7**, **12**, **17**, **18** and **21** inhibited NF-κB dependent transcription induced by PMA in a concentration-dependent manner. The dose effects for selected compounds **7**, **18** and **21** are shown in Fig. 2. The IC_{50} values are summarized in Table 2 and compared with the activity of enhyrin used as a positive control. None of the compounds inhibited Sp-1 dependent luciferase expression except **17**, indicating that the effect on NF-κB was also specific. Although structurally different phenylpropanoids from *Marrubium vulgare* (Labiatae) and *Scrophularia scorodonia* (Scrophulariaceae) have been reported as inhibitors of COX-2 (Sahpaz *et al.*, 2002; Diaz *et al.*, 2004) and NF-κB (Bremmer *et al.*, 2004); however, this is the first report of the action of phenylpropanoids from *Pimpinella* species on NF-κB. The results indicated that compounds with epoxyphenylpropanoids moiety (e.g. **12**, **17**, **18**, **21**) were more effective than compounds lacking the epoxy group (e.g. **7**). It was interesting to note that compound **19** did not show a similar effect. This could be due to *cis* configuration of a double bond compared with **18**. Similarly, compound **13** did not show any activity which could be due to the absence of a double bond in the two position compared

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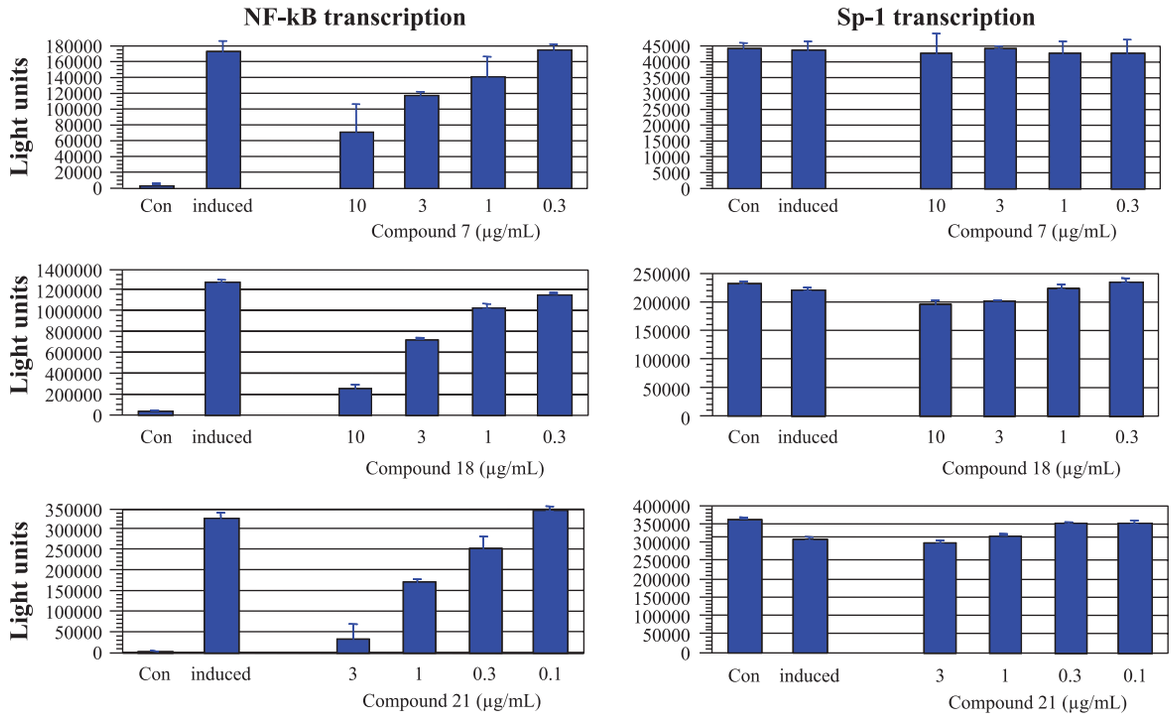

Figure 2. Effect of compounds **7**, **18** and **21** on NF-κB and Sp-1 mediated transcription in PMA induced SW1353 cells. As seen above, NF-κB mediated transcription is enhanced in PMA (70 ng/mL) induced cells versus control (uninduced) cells. Sp-1 is a control plasmid which is not induced by PMA treatment. Inhibition of NF-κB medicated transcription can be seen with increasing concentrations of compounds **7**, **18** and **21** while Sp-1 mediated transcription remained unaffected at similar concentrations (see Material and Methods for details).

Table 1. Inhibition of NF-κB mediated transcription in SW1353 cells by essential oils of *Pimpinella* species

Species	Plant part	Inhibition of luciferase activity (IC_{50} µg/mL)
P. anisetum Boiss. et Bal.	Fruits	NA
P. anisum L.	Fruits	>100
P. aurea DC.	Fruits	35
P. cappadocica Boiss. et Bal. var. *cappadocica*	Root	95
P. corymbosa Boiss.	Root	2
P. flabellifolia (Boiss.) Benth. et Hook ex Drude	Fruits	>100
P. isaurica Matthews	Aerial parts without fruits	70
P. kotschyana Boiss.	Fruits	47.5
P. nudicaulis Trautv.	Root	>100
P. olivieroides Boiss. et Haussk.	Root	>100
P. peregrina L.	Fruits	27.5
P. peucedanifolia Fisch. ex Ledeb.	Root	NA
P. puberula Boiss.	Fruits	55
P. rhodantha Boiss.	Root	6
P. saxifraga L.	Root	45
P. tragium Vill. *ssp. pseudotragium* (DC.) Matthews	Root	3
P. tragium Vill. ssp. *lithophila* (Schischkin) Tutin	Aerial parts without fruits	55
P. tragium Vill. ssp. *polyclada* (Boiss. et Heldr.) Tutin	Root	11

NA, no activity.

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Table 2. IC_{50} values of the inhibition of the NF-κB and Sp-1 mediated transcription in SW1353 cells by compounds 7, 12, 17, 18, 21

Compound	IC_{50} (μg/mL)	
	NF-κB	Sp-1
7	5.5	NA
12	1.2	NA
17	0.01	1.5
18	3.6	NA
21	1.1	NA
Enhydrin[a]	0.6	NA

NA, no activity.
[a] Positive control.

with **12**. These observations indicate that NF-κB inhibition activity of phenylpropanoids is specific and depends on the chemical nature of the compound. A minor chemical difference in the molecule seems to alter the activity. Compound **17** exhibited potent activity on NF-κB, it also inhibited Sp-1 activity indicating the nonspecific inhibition of luciferase activity. Compound **21** was isolated from the roots of *P. corymbosa* (most active amongst oils) and it showed the highest activity. Compounds **7** and **18** were isolated from aerial parts without fruits of *P. isaurica* Matthews and demonstrated strong activity. However, the activity of this oil was not very potent. The data suggest that the inhibition of the NF-κB mediated transcription by compounds **7**, **18**, and **21** might explain the beneficial effects of these plants in the treatment of inflammatory diseases and may be responsible for their antiinflammatory properties. Compounds **12** and **21** were also found to possess strong antimycobacterial activity (Tabanca *et al.*, 2003, 2005a). The antimycobacterial activity of the phenylpropanoids possessing an epoxide group and their mechanism of action was explained in our previous paper (Tabanca *et al.*, 2005a).

None of the isolated compounds showed any cytotoxicity to Vero cells or growth inhibition activity against human cancer cells (SK-MEL, SK-OV3, BT-549 and KB) (Data not shown).

In conclusion, the antiinflammatory activities of *Pimpinella* essential oils from different plant parts and isolated compounds were evaluated to obtain an insight into the beneficial effects of this plant species in conditions related to inflammation, reduced risk for cardiovascular diseases and cancer prevention by acting as antiinflammatory agents. Further studies are warranted to confirm the antiinflammatory activity of these compounds in more detail in animal models of inflammation.

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